Comparative Analyses of Continuous Data: the Need to Be Phylogenetically Correct

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ABSTRACT

In this paper I focus on the problem of non incorporating phylogenetic information when doing a comparative analysis. A review of the theory on this subject shows that not incorporating the phylogenetic information inflates the degree of freedom and can increase the risk of type I and type II errors of statistic tests done on cross species data (non phylogenetically controlled). The phylogenetic independent contrasts method (FELSENSTEIN, 1985) has been developed to resolve the problem of non-independence of data (*i.e.*, traits measured across different species) in comparative studies. After a presentation of the assumptions of this method, I provide one example on parasite species richness of mammals which shows the errors that lead to false conclusions. For example, a non phylogenetic approach (cross species comparisons) would lead to the conclusion that parasite diversity is linked to host body size, whereas a phylogenetic independent comparison shows no relationship between host body size and parasite richness. A non phylogenetic approach would thus lead us to reject the null hypothesis when it is false (Type I error). One assumption underlining the independent contrasts method is the random walk model (Brownian motion), which is used as a null hypothesis. Many traits that are considered in comparative studies are unlikely to be well described by a simple Brownian motion process. I propose to use MANTEL tests to detect evolutionary trends in comparative analyses. I performed a simulation that shows the efficiency of MANTEL tests for detecting evolutionary trends and for measuring phylogenetic effects. MANTEL tests could be one answer to the critical comments made on the independent contrasts method.

RÉSUMÉ

Analyse comparative des données continues : la nécessité d'être « phylogénétiquement correct »

Dans ce travail, je m'intéresse aux problèmes liés à la non prise en compte des informations phylogénétiques quant on réalise une analyse comparative. Une revue de la théorie concernant ce sujet montre que de ne pas incorporer les informations phylogénétiques augmente le degré de liberté et accroît les risques d'erreur de type I et de type II des tests statistiques effectués sur les données non contrôlées pour la phylogénie. La méthode des contrastes indépendants (FELSENSTEIN, 1985) a été développée pour résoudre le problème de la non-indépendance des données (les traits mesurés chez les différents taxons) dans les études comparatives. Après une présentation des hypothèses de cette méthode, je donne un exemple concernant les richesses parasitaires des mammifères terrestres qui montre les erreurs conduisant à des conclusions erronées. Ainsi, une approche non phylogénétique aurait conduit à la conclusion que la diversité parasitaire est liée à la taille de l'hôte, alors que la méthode des contrastes indépendants montre l'absence de relation entre ces deux variables. Une approche non phylogénétique peut conduire à rejeter l'hypothèse nulle alors qu'elle est vraie (erreur de type I). Une des hypothèses de la méthode des contrastes indépendants est le modèle de marche aléatoire (mouvement brownien). De nombreux traits, pris en compte dans les analyses comparatives, ne sont pas bien décrit par le modèle de mouvement brownien. Je propose d'utiliser

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les tests de Mantel pour détecter les tendances évolutives dans les analyses comparatives. J'ai conduit une simulation qui montre l'efficacité des tests de Mantel pour détecter les tendances évolutives et mesurer les effets phylogénétiques. Les tests de Mantel peuvent être une des réponses aux critiques effectuées sur la méthode des contrastes indépendants.

INTRODUCTION

There are two ways for analyzing evolutionary processes. The first one, the population approach, focuses on micro-evolutionary processes and tries to find adaptation at work, *i.e.* the evolution of a specific character under natural selection or sexual selection. The second one, the comparative method, tries to identify adaptation by studying the evolution of a specific character, in different lineages, supposed to be driven by the same selection pressures. The development of cladistic analyses has challenged the definition of adaptation. For example, CODDINGTON (1988) has defined an adaptation as an apomorphic function promoted by natural selection. I will concentrate on the second approach.

First of all, we have to distinguish the differences between phylogenetic effects from phylogenetic constraints. DERRICKSON & RICKLEFS (1988) have drawn the attention on the fact that numerous biologists do not make the difference between phylogenetic effects and phylogenetic constraints. According to these authors, the phylogenetic effects are only the expression of the tendency of related species to be similar because they share a common history. They defined a phylogenetic constraint as the effect of history onto the changes in diversification of a given clade or as the differences in evolutionary interactions between a phenotype and its environment. However, as emphasized by MCKITRICK (1993) such definition refers more to the results than to the causes of a constraint. MCKITRICK (1993) suggested that a constraint highlights the absence of a given character or the lack of an expected evolution. She proposed the following definition where a phylogenetic constraint is "any result or component of the phylogenetic history of a lineage that prevents and anticipated course of evolution in that lineage". The lack of viviparity among birds is an example of phylogenetic constraint.

Very early, people have recognized several pitfalls linked with cross-species comparisons. It has been recognized that taxonomic relationships greatly influence the correlation between the analyzed traits (STEARNS, 1992). Interspecific comparison is a very common approach in ecology (as well as in other branches of biology). Many recent studies, and even recent textbooks, in ecology or evolutionary biology continue to ignore these statistical pitfalls and persevere to ignore the importance of the phylogeny and the history of organisms.

Some evolutionary biologists use parsimony methods for inferring the evolution of a particular character. GARLAND & ARNOLD (1994) argued that the application of parsimony analyses can be justified only on methodological grounds but do not refer to any model of evolution (but see SOBER, 1994 for the use of parsimony in evolutionary biology). FELSENSTEIN (1988) challenged the view that reconstructing phylogenies is a statistical problem and implies an explicit model of evolution. People interested in the evolution of discrete characters mostly use parsimony analyses whereas those dealing with continuous characters use independent comparative methods (but see PAGEL, 1994).

It is not my aim to compare these two very different methods (parsimony *versus* independent comparative method) for the analysis of adaptation. Rather, I focus deliberately on the statistical approach in order: (1) to convince evolutionary ecologists about the need to control for phylogeny when comparing different species, (2) to draw the attention of

phylogeneticists to models (and statistics) that underline every methods, (3) to propose MANTEL tests as a method to detect evolutionary trend.

HOW TO REVEAL PHYLOGENETIC EFFECTS? A FIRST APPROACH

FISHER & CHAPMAN (1993) tried to answer to this question by analyzing the dispersal mechanisms of plant fruit. The objectives of their study were to examine the degree to which plants have evolved predictable, disperser-specific syndromes and to determine the consequences of using different taxa as sampling units when analyzing comparative data to test for the existence of dispersal syndromes. These authors recognized that using species as independent sample units implies that the analyzed character (fruit morphology) should have evolved independently in any clade, which is not self-evident. Furthermore, an analysis based on species will dramatically inflate the number of events. In the absence of a fully resolved phylogeny, FISHER & CHAPMAN (1993) proposed to use genera as sample units. The hypothesis is that if the apparition of a given trait is the result of convergent evolution then this correlation should always be found when using genera as sample units. Because the correlation was lost using genera as sample units, FISHER & CHAPMAN (1993) concluded that a study based at the species level is not unbiased. This example highlights two major problems. First, the use of taxonomic information is arbitrarily and, second, the use of species as independent points may lead to false conclusion.

WHY USING PHYLOGENETIC INFORMATION IN COMPARATIVE ANALYSES?

Three pitfalls should be avoided in comparative analyses:
(1) not incorporating phylogenetic information may inflate the degrees of freedom,
(2) high risk of rejecting H_0 when it is true (Type I error),
(3) high risk of accepting H_0 when it is false (type II error).

Not incorporating phylogenetic information implies that we make the assumption of a true case of multiway speciation events ("hard polytomies"; MADDISON, 1989), which refers to a star phylogeny. However, most phylogenies are dichotomous even if some parts are unresolved (soft phylogeny). Imagine the case of 5 species, a star phylogeny gives (5-2=3) degrees of freedom while a dichotomous phylogeny gives (5-3=2) degrees of freedom or less (GARLAND & ARNOLD, 1994).

Figure 1, redrawn from GITTLEMAN & LUH (1992), shows the problem of phylogenetic relations. Suppose a known phylogeny with 2 genera and 6 species. By plotting trait variations and ignoring phylogenetic pattern we might find a relationship whereas it is erroneous (type I error: false rejection of H_0). Conversely, we might reject a relationship (type II error: false acceptation of H_0) which actually exists.

I will give below an example showing both statistical errors.

THE INDEPENDENT CONTRASTS METHOD

The phylogenetic independent contrasts method (FELSENSTEIN, 1985; MARTINS & GARLAND, 1991; PAGEL, 1992; GARLAND, 1992) has been developed to resolve the problem of non-independence of data (*i.e.* traits measured across different species) in comparative studies. FELSENSTEIN (1985) suggested a procedure for calculating comparisons between pairs of taxa at each bifurcation in a known phylogeny (Fig. 2).

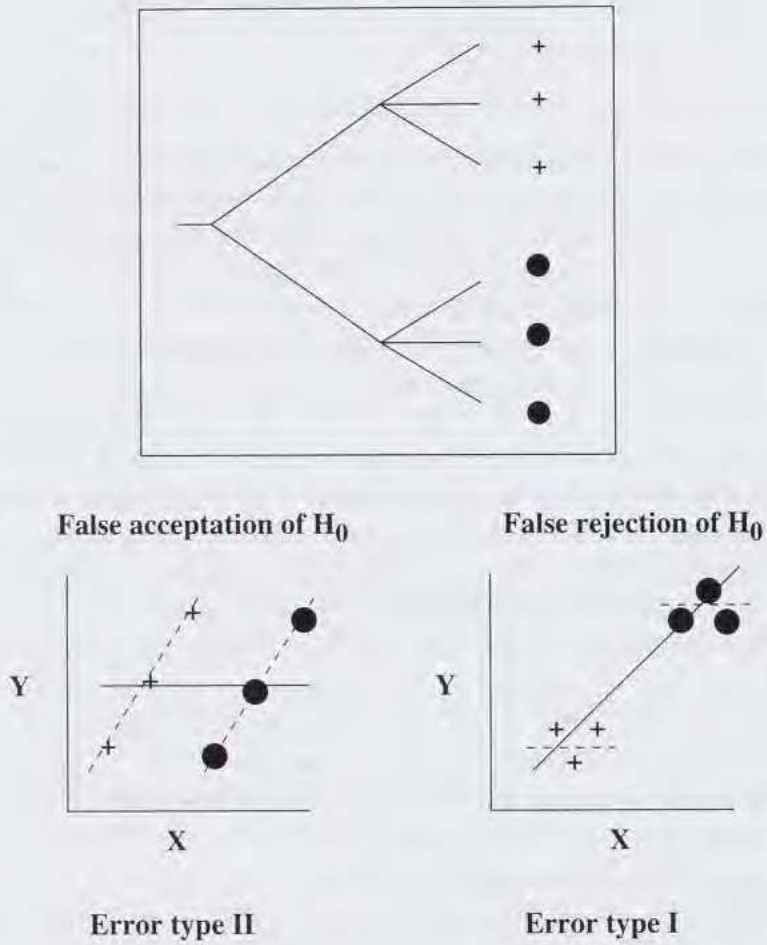
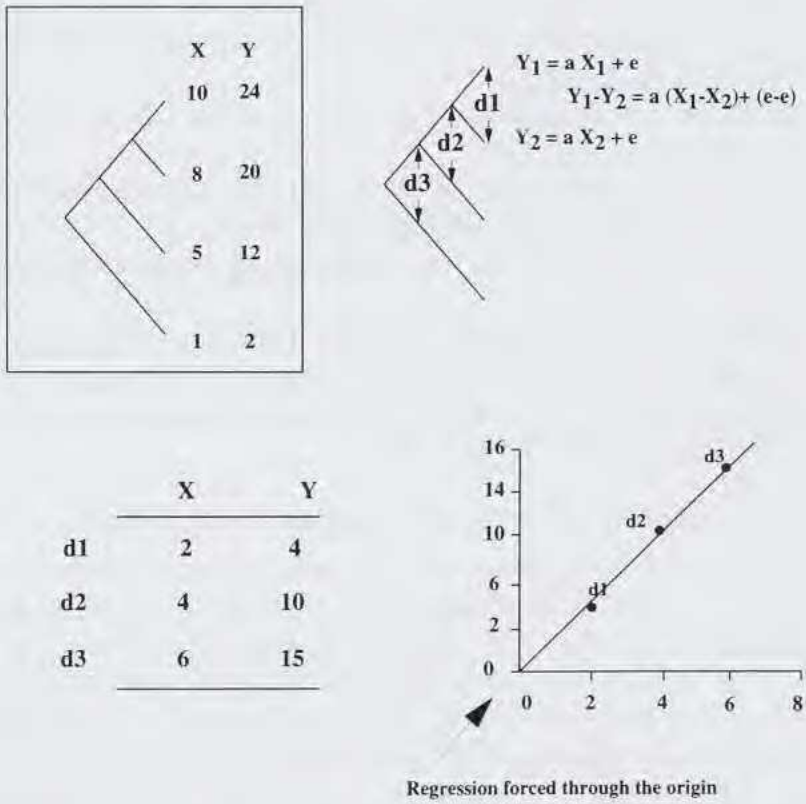


FIG. 1. — Ignoring phylogenetic relationships may lead to erroneous conclusions. A Type I error (false acceptation of the null hypothesis) occurs when rejecting the extant correlations (dashed lines) whereas a Type II error (false rejection of the null hypothesis) occurs when claiming correlation (solid line) when its actually false (dashed lines). The illustration is after GITTLEMAN & LUH (1992).

In a phylogenetic tree, the independent events (on which an analysis can be performed) correspond to the nodes that give rise to daughter branches. For each branch of a node, values for a given variable are obtained by averaging the values of its own daughter branches. Then the difference for each variable between the two daughter branches of each node is calculated. In the calculation of contrasts, the direction of subtraction is arbitrary. Multiple nodes can be treated in a way that gives a single contrast (PURVIS & GARLAND, 1993). Pairs of sister branches that diverged a long time ago are likely to give greater contrasts than pairs of sister branches that diverged recently. It is thus necessary to standardize each contrast through division by its standard deviation where the standard deviation of a contrast is the square root of the sum of its branch lengths (GARLAND *et al.*, 1992). In the absence of information on branch length, one can assume each branch length to be equal to unity. Another method is proposed by GRAFEN (1989) for assigning arbitrary lengths. In this method the age of a node is assigned as the number of

daughter groups descended from that node minus one. Nevertheless, GARLAND *et al.* (1992) showed that using arbitrary or real branch lengths often leads to similar results. In order to check that contrasts are properly standardized it is suggested to perform a regression of the absolute values of standardized contrasts versus their standard deviations. In case of positive relationship it is necessary to transform branch lengths before computing standard deviations (GARLAND *et al.*, 1992). All correlations between contrasts are forced through the origin (Fig. 2).



	X	Y
d1	2	4
d2	4	10
d3	6	15

FIG. 2. — The independent contrasts method. The illustration is after GITTLEMAN & LUH (1992) and PURVIS & RAMBAUT (1995).

The three main assumptions of independent contrasts are:
(1) a correct topology,
(2) branch lengths measured in units of expected variance of character evolution,
(3) a Brownian motion model of character evolution or random walk model (FELSENSTEIN, 1985; 1988).

Under a Brownian motion model of evolution, a change in the mean phenotype is expected to be non-directional and to occur at a constant rate. This rate can be described in terms of the relation between the variance among species phenotypes and time as:

$$V_b = \beta t + \varepsilon$$

As pointed out by MARTINS (1994), many traits that are considered in comparative studies are thought to have been the subject to the action of natural or sexual selection. Thus, these traits are unlikely to be well described by a simple Brownian motion process. The performances of the independent contrasts under different models of character evolution have been tested (see MARTINS & GARLAND, 1991; MARTINS, 1994; BJÖRKLUND, 1994). Simulation studies indicate that the independent contrasts method produces acceptable error rates. Moreover, the independent contrasts method produces less error rates than other phylogenetic correction methods, like nested ANOVA or phylogenetical autocorrelation (MARTINS & GARLAND, 1991; PURVIS *et al.*, 1994; DIAZ-URIARTE & GARLAND, 1996).

Three statistical assumptions must be tested when working with a real data set (GARLAND *et al.*, 1992; PURVIS & RAMBAUT, 1995):

(1) the random walk model can be tested by regressing the absolute values of the standardized contrasts against the estimated nodal values,

(2) homogeneity of variances can be tested by regressing the absolute values of the standardized contrasts against the height or ages of the corresponding nodes,

(3) and ANOVA can be used to test for heterogeneity of variances amongst multiple node values.

However, one problem with the independent contrasts method is the accurate estimation of the ancestral values at ancestral nodes (PAGEL, 1992). The method of averaging values can introduce several biases. Excluding ancestral nodes from the analysis is one way to test if the relationship remains identical with actual species (PAGEL, 1992).

PARASITE RICHNESS OF MAMMALS AS EXAMPLE

I compiled data on nematodes recovered from 66 species of terrestrial mammals. These data were collected from several sources based on a survey of 90 studies published over the last 30 years. Comparative analyses of parasite species richness should avoid 2 pitfalls: sample size (GREGORY, 1990; WALTHER *et al.*, 1995) and phylogenetic confounding effects (HARVEY, 1996). As GREGORY (1990) and WALTHER *et al.* (1995) pointed out, investigations on parasite species richness must take into account differential sampling effort. Differential sampling effort is a consequence of both the researcher's sampling procedure and of the geographical range of the hosts, and both may affect host and researcher encounters, and thus directly influences the observed number of parasite species.

The need to take the phylogeny into account is related to the coevolution between hosts and parasites. Hence, host phylogeny may be important in determining the richness of a parasite community (HOLMES & PRICE, 1980; BROOKS & MCLENNAN, 1991). Furthermore, cross-species comparisons performed using species values as independent data points may be confounded by the phylogenetic relationship of the analyzed species (FELSENSTEIN, 1985; HARVEY & PAGEL, 1991; MARTINS & GARLAND, 1991). For example, a correlation between host body size and parasite species richness may arise because a group of related and same-sized hosts have a high parasite species richness because of their common phylogenetic origin and not because of common ecological forces. Closely related species tend to be similar. Therefore, species values cannot be treated as statistically independent points (HARVEY & PAGEL, 1991).

I based the analysis on the working phylogeny of mammals (Fig. 3) proposed by POULIN

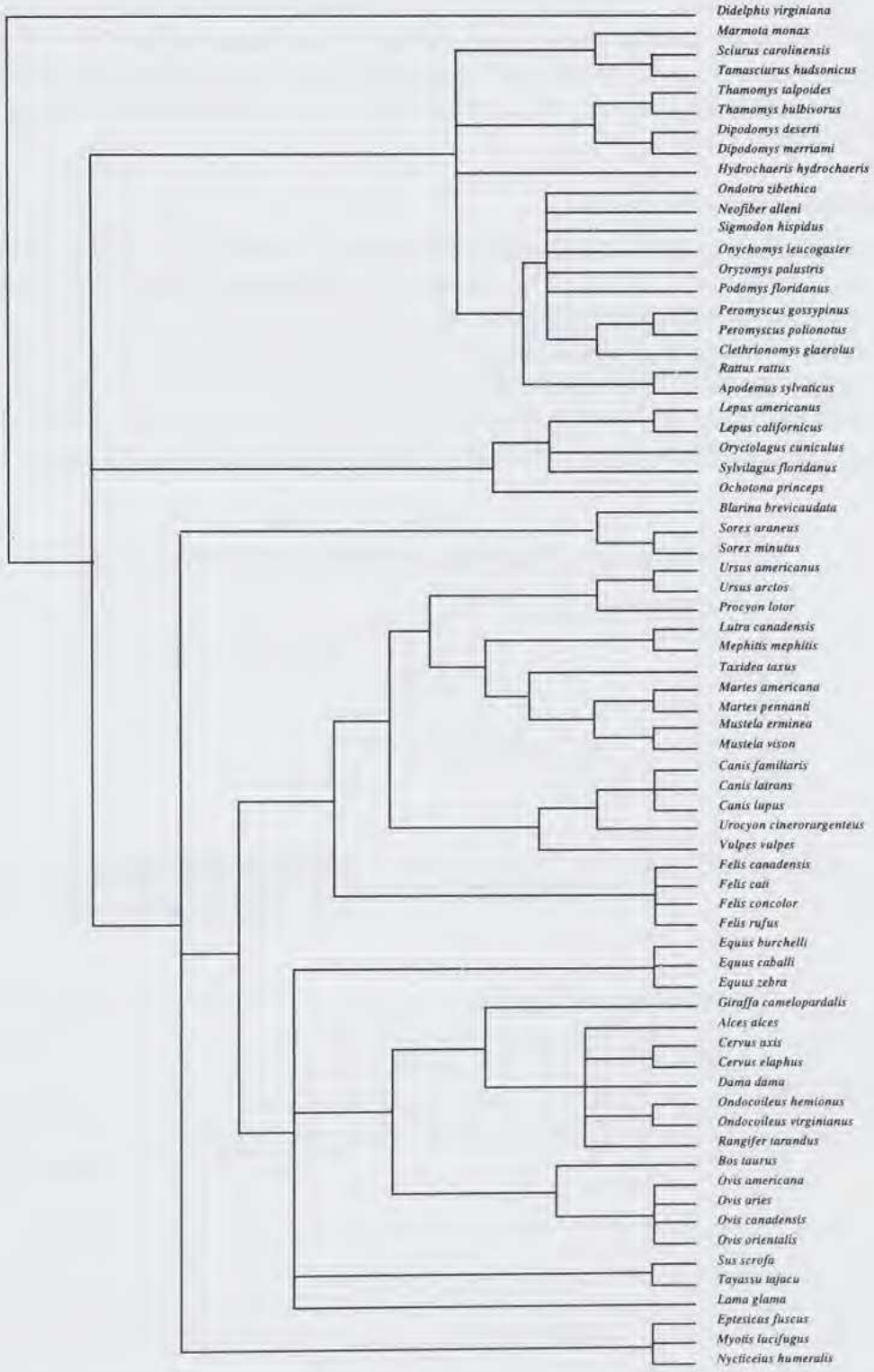


FIG. 3. — Phylogeny of mammals used in the analysis (this phylogeny redrawn from POULIN, 1995 is based on various sources: molecular and morphological data)

(1995). I used the C.A.I.C. program (PURVIS & RAMBAUT, 1995). Data on parasite species richness and host body lengths were logarithmically transformed (HARVEY, 1982). Because parasite species richness can correlate with sampling effort, both variables were controlled for host sample size before the analyses. All correlations between contrasts were forced through the origin (GARLAND *et al.*, 1992).

Parasite richness and host body size

Cross species analysis and phylogenetic independent method gave rise to different results (Fig. 4). A non phylogenetic approach (cross species comparisons) leads to the conclusion that parasite diversity is linked to host body size. However, a phylogenetic independent comparison of contrasts analysis showed no relationship between host body size and parasite richness. A non phylogenetic approach would lead us to accept the null hypothesis when it is false (Type I error). My results support those of POULIN (1995) who also did not find any relationship between mammal body size and parasite species richness when correcting for host phylogeny.

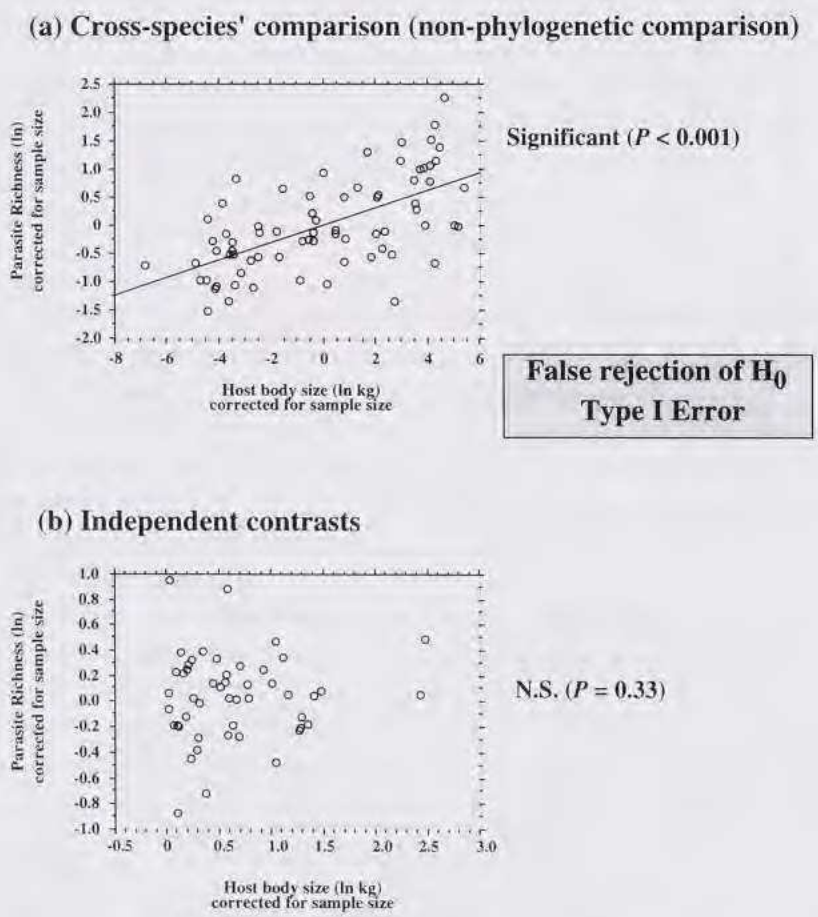


FIG. 4. — A significant relationship between host body size and parasite diversity (nematodes) is found when using a non-phylogenetic approach whereas it is false as detected by the independent contrasts method. Parasite species richness is controlled for sampling effort.

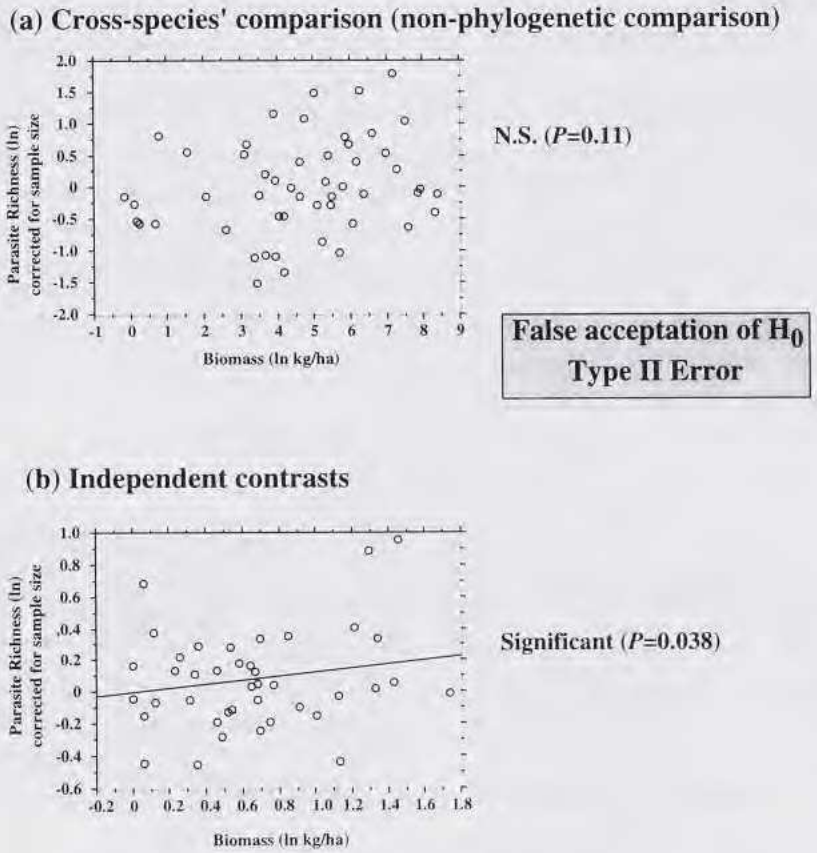


FIG. 5. — A lack of relationship between host biomass and parasite diversity (nematodes) is found using a non-phylogenetic approach whereas the independent contrasts method detects a positive relationship.

Parasite richness and host biomass

The results found by the two methods were also different. While a non-phylogenetic approach did not detect any relationship between the two variables (Fig. 5), the independent comparison allows to find a significant relationship between nematode diversity and host biomass. Thus, a non phylogenetic approach will lead to accept the null hypothesis whereas the null hypothesis is wrong (Type II error).

DETECTING EVOLUTIONARY TRENDS AND THE USE OF MANTEL TESTS

Analyzing evolutionary trends was the topic of the essay of MCKINNEY (1990), who proposed time series analyses as a tool for detecting an evolutionary trend. For McKinney, trends are persistent statistical tendencies in some variables (such as morphological) in an evolutionary time span. De facto, random walk (Brownian motion) is used as a null hypothesis. MCSHEA (1994) argued that large-scale evolutionary trends may be passive or driven. Whereas the passive trend may correspond to a Brownian motion of character evolution (random walk), the driven trend corresponds to a selection-driven system (MCSHEA, 1994).

Both systems of evolution (passive or driven) yield to the conclusion that related species share the same characters due to their phylogenetic proximities. However, in a passive system distant species can share the same characters because of the random evolution of characters (Brownian motion).

I performed a simulation study, to show that MANTEL tests cannot detect pure Brownian motion of character evolution (passive trends) but can detect driven evolutionary trends with acceptable error rates. MANTEL tests have been used to quantify phylogenetic effects (TAYLOR & GOTELLI, 1994), and an extended version of this test has been proposed by LEGENDRE *et al.* (1995). However, the robustness of the MANTEL test in comparative analyses has not yet been evaluated.

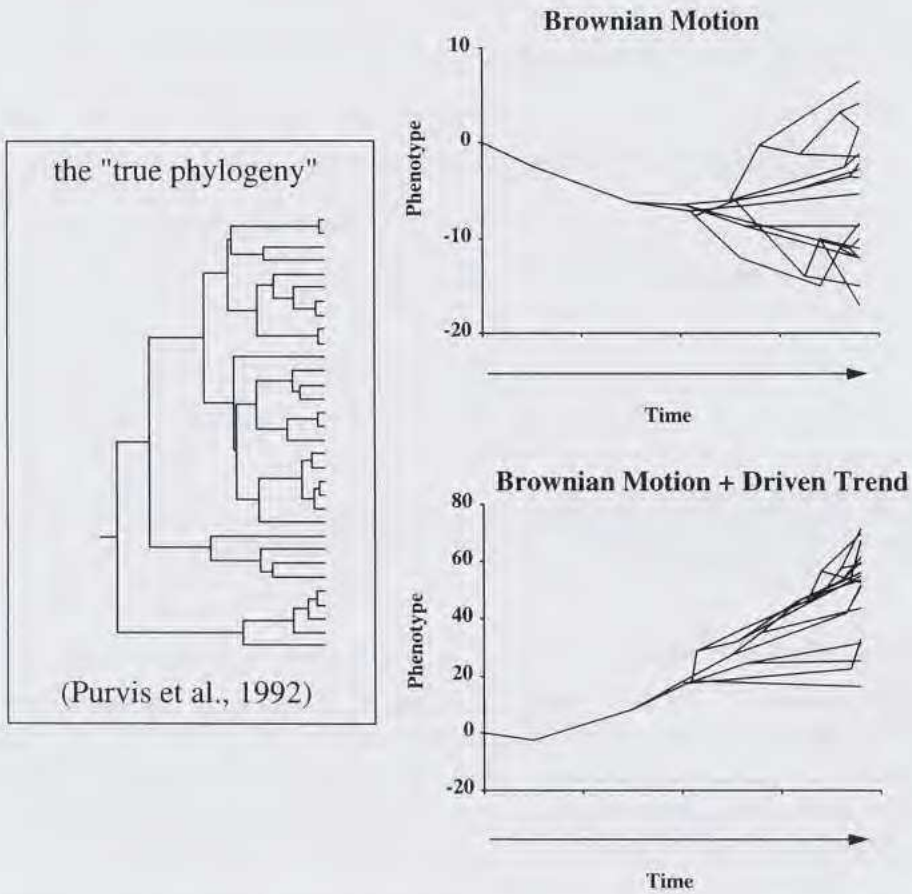


FIG. 6. — The "true phylogeny" used in the simulation study. The changes in variance among species phenotypes with time are shown under a Brownian model of evolutionary change (with $\sigma^2 = 1$ throughout clade) and under a Brownian + a driven evolutionary trend.

Methodology and examples

Using a modified version of PURVIS *et al.*'s methodology (1994) to take into account a driven trend, values of pairs of characters, Y and X, were generated for the 32 species along the phylogeny given in Fig. 6. For each branch segment, the changes of values of these traits are given by:

$$\Delta X = N(0,1)^* \sqrt{branch\ length} + pi(\beta)$$

$$\Delta Y = a.\Delta X + (1 - a).N(0,1)^* \sqrt{branch\ length}$$

where N(0,1) is a normal pseudo-random number of mean 0 and variance 1, a is the input correlation and pi(β) the probability of increase (see below). Each normal random number is multiplied by the square root of the branch length (following PURVIS *et al.*, 1994). Starting from the root of the tree, where X = 0 and Y = 0, values at successive nodes i are computed as

$$X(i+1) = X(i) + \Delta X$$

$$Y(i+1) = Y(i) + \Delta Y$$

The values of X and Y for the species, located at the tip of the branches, were calculated by summing the changes along all branches of the phylogeny.

In a passive system (pure Brownian motion), pi = 0. In a driven system, the value b (10 in my simulations) is added to ΔX according to a probability of increase pi (pi = 0.9; I used the same value as in MCSHEA, 1994). The passive system corresponds to the simulation method of PURVIS *et al.* (1994) whereas the driven system follows a similar methodology to that exemplified by MCSHEA (1994).

I calculated 1000 pairs of X variable with a = 0 and used them for detecting errors of

	Mantel tests	
	X and Phylogeny	Y and Phylogeny
Pure Brownian		
test of validy (Type I)	p>0.05	p>0.05
test of power (Type II)	p>0.05	p>0.05
Brownian + Driven trend		
test of validy (Type I)	p<0.05	p>0.05
test of power (Type II)	p<0.05	p>0.05

FIG. 7. — Mantel test method. In Mantel tests, the X variable is transformed into distance matrix **X**, by computing the "distance" among values (absolute value of the difference). The phylogeny is represented by a matrix **P** of patristic distances among species. Patristic distances are computed as the lengths of segments along the evolutionary tree that separate two species. The regression of the individual values in the matrices yields the regression coefficients constructed by Monte Carlo simulation (MANLY, 1991). The significance (p) was determined by Monte Carlo simulation.

Type II. Similarly, I used a further set of 1000 pairs with a value for $a = 0.3$ for detecting errors of Type I (I used the same value as PURVIS *et al.*, 1994).

In Mantel tests, the X and Y variables are transformed into distance matrices **X** and **Y**, by computing the "distance" among values (absolute value of the difference). The phylogeny is

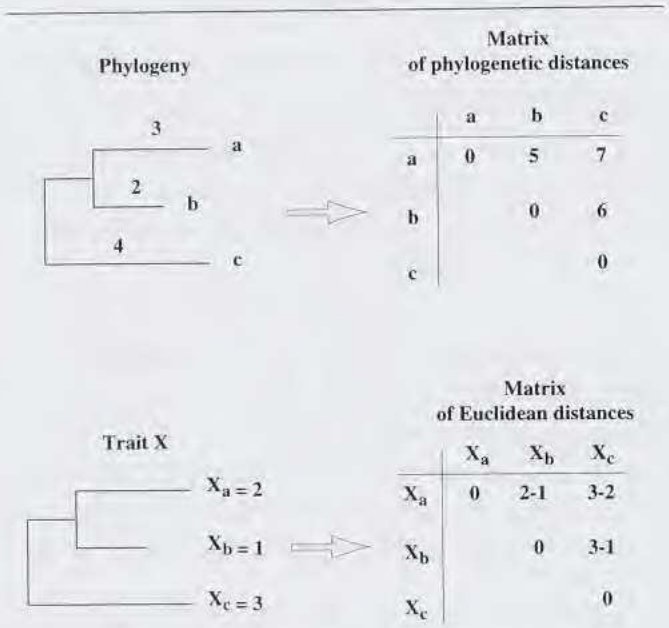


FIG. 8. — Results of the simulation study for a passive system (Brownian motion of character evolution) and a driven system (phylogenetic trend). Test of validity (detection of type I errors) is carried out using a fixed input correlation of $a = 0$; Test of power (detection of type II) is performed using a fixed input correlation of $a = 3$. Mantel tests were done between variable X and the matrix of the phylogeny (999 permutations each for the MANTEL test).

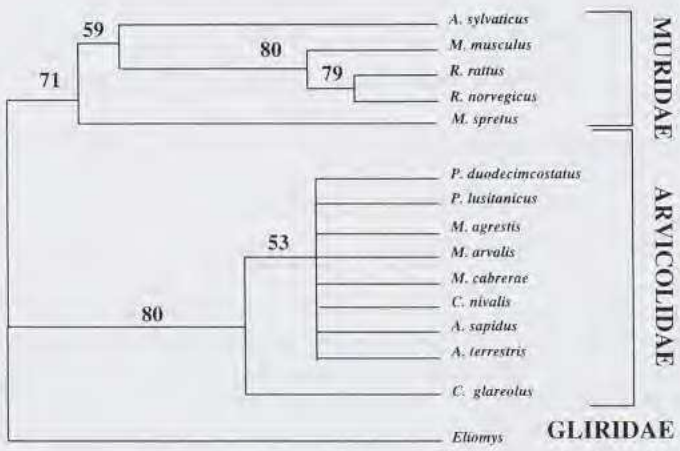


FIG. 9. — Working phylogeny of rodents. Evolutionary divergences between rodents were obtained from various sources: paleontological records, morphological and molecular data. (see FELIU *et al.*, 1997).

represented by a matrix **P** of patristic distances among species. Patristic distances are computed as the lengths of segments along the evolutionary tree that separate two species (Fig. 7).

I implemented the Mantel test according to MANLY (1991). The regression of the individual values in the matrices yields the regression coefficients constructed by Monte Carlo simulation (SMOUSE *et al.*, 1986; MANLY, 1991). The significance (p) was determined by Monte Carlo simulation (999 replications) (LEGENDRE *et al.*, 1995).

According to my hypothesis, Mantel tests cannot detect a passive trend but can detect a driven trend based upon both validity (a = 0) and power tests (a = 0.3) (Fig. 8). The detection is found only for the X variable, which was the variable affected by the driven trend. Based upon these results, it may be possible to detect a phylogenetic trend in comparative analyses. This can be seen in the following real data sets: the parasite species richness of Iberian rodents and the parasite species richness of African cyprinids. Using data on parasites of rodents, collected over an eighteen year period on the Iberian peninsula, FELIU *et al.* (1997) investigated the determinants of parasite species richness in Iberian rodents. More than 70 species of helminth parasites (nematodes, cestodes and digenes) were identified among fifteen species of rodents, for which a working phylogeny has been proposed (Fig. 9). Parasites were classified into groups according to their host specificity. Specificity corresponds to the number of infected host species by a given parasite species: the larger the host species number, the lower the specificity. One explanation of parasite species richness is linked to host phylogeny. A Mantel test shows that richness of specific parasites (corrected for host sample size according to WALTHER *et al.*, 1995)

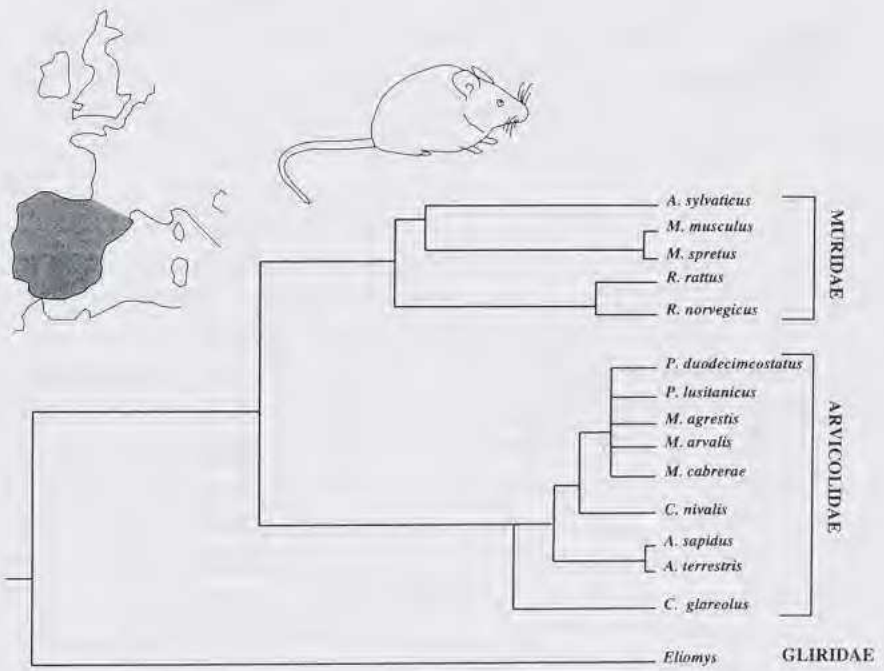


FIG. 10. — Relationship between rodents using parasites as characters in a parsimonious construction tree (FELIU *et al.*, 1997). Specific parasite species are coded as characters (values of bootstrap analysis are given on the figure, 100 replicates). Note that major phylogenetic relationships are found.

is correlated with the phylogeny of their host ($p = 0.001$, $R = 0.66$). This pattern is clearly illustrated when using parasite species as characters for a tree reconstructing host relationships (Fig. 10). The obtained consensus tree reflects the major phylogenetic divisions of the host group. Thus, the detection of a phylogenetic trend, the increase of parasite species richness through the diversification of their hosts, is revealed by MANTEL tests and confirmed by tree reconstruction.

GUÉGAN *et al.* (1992) investigated the richness of monogeneans (ectoparasites) of cyprinid fishes and found that host length is a major determinant of ectoparasite diversity. More recently, GUÉGAN & MORAND (1996) have shown using the independent contrasts method that parasite species richness is correlated with changes in the level of host ploidy. Because of the loss of explanatory power (percentage of variance) when using independent comparison, we may suggest that history of the host group can partially explain parasite species richness. In this case, I used a MANTEL test (Fig. 11) and found that phylogeny effectively explains a substantial amount of variance of species richness ($p < 0.001$; $R = 0.16$). In other words, this finding suggests that related species of hosts tend to have the same parasite species richness because most of the parasites have been inherited from their common ancestors.

These two examples illustrate how Mantel tests can be applied in comparative analyses. However, I would like to emphasize that the lack of detection of a phylogenetic correlation does

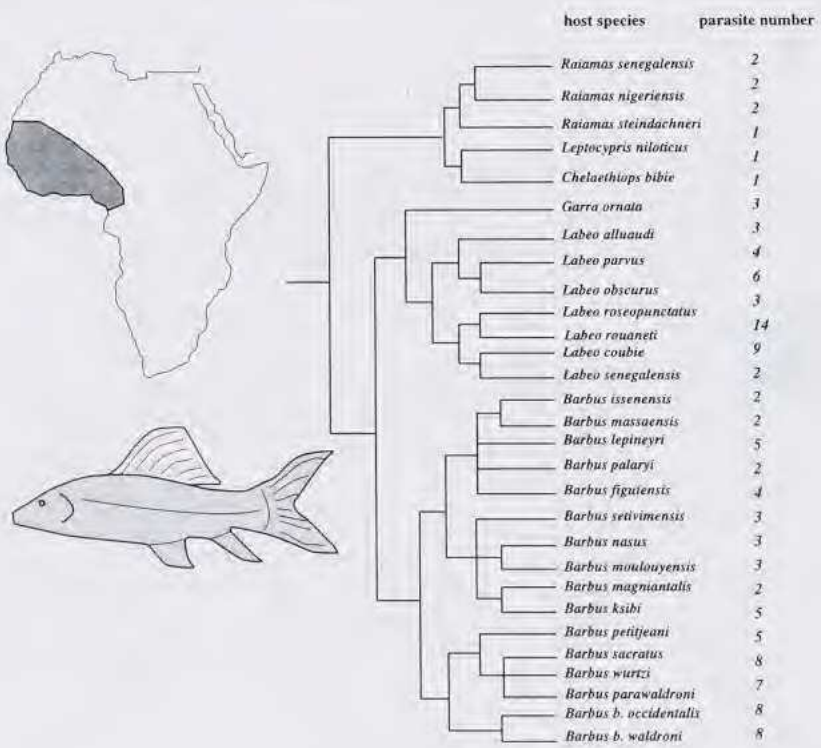


FIG. 11. — Phylogeny of African cyprinid fish based on isoenzymes data (from GUÉGAN & MORAND, 1996) with number of parasite species.

not allow to the conclusion of the absence of phylogenetic effects. The simulation studies clearly show that Mantel tests do not detect passive evolutionary trends (pure Brownian motion of character evolution) and that comparative studies should always use the independent contrasts method.

SKEPTICISM ABOUT COMPARATIVE METHODS?

Before concluding, it is necessary to mention some problems concerning the use of comparative methods in evolutionary biology. Two different criticisms have been put forward, one by LEROI *et al.* (1994) and the other one by WESTOBY *et al.* (1995a, 1995b).

LEROI *et al.* (1994) argued that comparative methods are "valuable for examining the evolutionary history of traits but they will often mislead in the study of adaptive processes". Their major concern is that we know very little on the evolutionary genetic mechanisms responsible for distributions of traits among species. They claimed that it is very difficult to justify any evolutionary scenario without evidence of historical selection forces and, more important, the genetic relations among traits. Some of their arguments concern mainly the invocation of constraints in the explanation of either adaptation or phylogenetic conservatism. However, the problem is more a problem of definition (what is a phylogenetic constraint) than a problem of method (the use of comparative method). A second set of arguments addresses the question of the evolution of continuous characters, the topic of this study. Using the example of the scaling of brain and body size, described as a power function, they found at least two problems of the comparative method. The first is that of confounding selection pressures. I cannot see why this is a specific problem of the comparative method. A correlation constitutes no proof whether the correlation is the result of the comparative method or any other methods. The second criticism deals with "the confounding of the causal influence of selection with that of genetic correlations". This is a more serious critique but, again, the problem is more related to the causes and correlations than to methods. Indeed, LEROI *et al.* (1994) concluded their study with the acknowledgment "that the methods of comparative biology and genetics might be usefully combined".

The second criticism came from WESTOBY *et al.* (1995a, 1995b). Their concern was that a phylogenetic correction (i.e. phylogenetic analysis) is not a correction but rather a conceptual decision which gives priority to one interpretation over another. In fact, they assumed that part of variation of a given trait is correlated with phylogeny and other part correlated with ecology. However, their arguments refer to the notion of phylogenetic niche conservatism. This process can be described as follows: "the ancestor of a lineage possesses a constellation of traits, enabling it to succeed in a particular habitat and disturbance regime, through a particular life history and physiology. The lineage will therefore leave most descendants in similar niches. This niche conservatism in turn will tend to sustain a similar constellation of traits in descendants of the lineage (WESTOBY *et al.*, 1995a). HARVEY *et al.* (1995) gave a clear answer to that questions by emphasizing that the independent contrasts method does not remove phylogenetic effects but produces plots in which all the variation of the data set in one variable is graphed against all the variation in the other variable. In this way, phylogenetic niche conservatism means that adaptations to different components of the niche will be correlated (HARVEY *et al.*, 1995), which is what the contrasts method has been designed to detect.

CONCLUSION

Within a multi-species study, species do not necessarily represent independent data points (KELLY & PURVIS, 1993; HARVEY, 1996). The recent debate involving WESTOBY *et al.* (1995a, 1995b) and HARVEY *et al.* (1995) highlighted some misinterpretations of comparative methods.

Comparative biologists have drawn attention to all the biases which could arise when the phylogenetic information are not taken into account (PAGEL & HARVEY, 1991; GARLAND *et al.*, 1992; MARTINS, 1995; HARVEY, 1996). Moreover, as emphasized by GARLAND & ARNOLD (1994), caution should be exerted to all comparisons involving only two species (there is no degree of freedom!).

In this study, I have provided one example on parasite species richness of mammals which showed these biases. Not incorporating phylogenetic information would have lead to false conclusions.

The independent contrasts method remains the best method to avoid the phylogenetic confounding effects (HARVEY, 1996, but see BJÖRKLUND, 1994, for a comparison of this method with character mapping by optimization on a cladogram). Even if the independent contrasts method assumes a model of character evolution (the Brownian motion model or any other models, see Martins, 1994), simulation studies showed that this method is very robust (low error rates). However, without a correct phylogeny of the studied organisms it is impossible to test evolutionary hypotheses. The main problem is the availability of a correct phylogeny. Recently LOSOS (1994) proposed to use computer simulations to generate a large sample of possible phylogenies in the absence of a correct topology and to calculate independent contrasts for each generated tree. LOSOS (1994) gave two rules of thumb. First, if all analyses give the same result (significant or not), then the result is independent of what the true phylogeny is. Second, if a substantial minority of phylogenies yield different results from the majority, then the outcome of the analysis will depend on the correct phylogeny.

There are some other methods in comparative analyses which solve the problem of non-independence (LYNCH, 1991), for example, the phylogenetic autocorrelation method (GITTLEMAN & KOT, 1990) or the permutation on distance matrices method (LEGENDRE *et al.*, 1995; MORAND, 1996; MORAND *et al.*, 1996). All these other methods have not been tested for their power in a wide range of character evolution (but see PURVIS *et al.*, 1994; MARTINS, 1995). I carried a simulation study showing the efficiency of Mantel tests for detecting evolutionary trends and for measuring the phylogenetic effect. I hope that Mantel tests will be an answer to the questions of WESTOBY *et al.* (1995). Mantel tests done on the data set (each variable against the phylogeny) will indicate if there is a trend in the changes of the values of each variable. We should remember that the lack of correlation may not lead to the conclusion of the independence of species. A correlation may indicate that the character does not evolve under a pure Brownian motion. The Mantel tests reveals a phylogenetic niche conservatism or, in the case of parasite diversity, a phylogenetic trend but they do not allow to avoid a phylogenetic independent analysis.

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